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ANTIMICROBIAL and ANTI-INFLAMMATORY PROPERTIES OF *Garcinia indica* Choisy FRUIT EXTRACTS AGAINST UROPATHOGENIC BACTERIA

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Introduction

With millions of cases each year, Urinary Tract Infections (UTIs) are among the most commonly diagnosed illnesses worldwide. The disease refers to a variety of illnesses that affect the kidneys, ureters, bladder, and urethra, which are all parts of the urinary system. In addition to having a major negative influence on a person's quality of life, these infections can cause major side effects like kidney damage or systemic infections like sepsis if left untreated. Because of their high frequency and related medical expenses, UTIs are especially important in the field of medicine. They are the cause of many hospital stays, outpatient visits, and prescriptions for antibiotics. It is essential to comprehend UTIs in order to create preventative and treatment plans that work. It is caused by often bacteria, invading and growing within the urinary system. In order to maintain the body's internal balance of water and electrolytes while eliminating waste, urine must be produced, stored, and expelled by the urinary system, which is made up of the kidneys, ureters, bladder, and urethra. The lower urinary tract, which includes the bladder (cystitis) and urethra (urethritis), is where UTIs most commonly occur, however they can affect any region of this system. The infection may occasionally spread to the kidneys, where it can result in pyelonephritis, a more serious illness(O'Brien et al., 2016).

In addition to their great frequency, UTIs are significant because, if ignored, they can result in significant discomfort and consequences. They are among the most prevalent infections in the world, affecting people of all ages, genders, and socioeconomic backgrounds. According to studies, over 70% of women will get at least one UTI in their lives, meaning that women are disproportionately afflicted. The main cause of this increased incidence in women is anatomical characteristics, including as the urethra's shorter length and its closeness to the anus, which allow microbes to easily enter the urinary tract (Abou Heidar et al., 2019).

Although the majority of UTIs are simple and may be resolved with the right care, they are a serious public health problem because of their propensity to develop into more serious illnesses including kidney infections or bloodstream infections (urosepsis). Early detection and efficient therapy are crucial since these complications might result in long-term health problems such chronic renal disease and systemic organ dysfunction.

Pathogenesis

The majority of UTIs are caused by bacteria, with *Escherichia coli (E. coli)*, particularly uropathogenic *E. coli* (UPEC), being the predominant pathogen in up to 80-90% of cases. Other bacterial species involved include *Klebsiella pneumoniae*, *Proteus mirabilis, Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa* (more common in complicated UTIs). In certain populations, such as immunocompromised individuals, fungi (e.g., Candida species) and viruses can also be causative agents. These organisms exploit weaknesses in the host's defences, leading to infection. Uropathogenic *E. coli* is the main cause of both simple and complex UTIs (UPEC). *E. coli (E. coli)* are the predominant pathogen in simple CAUTIs, followed by *Proteus species, Staphylococcus saprophyticus, Klebsiella species*, and other Enterobacteriacea. The uropathogen *E. coli* is closely followed by Enterecoccous species, *K. pneumoniae, Candida species, S. aureus, P. mirabilis, P. aeruginosa*, and group B streptococci in patients with complicating conditions such diabetes, spinal injuries, and other co-morbidities. Both upper and lower UTIs have a comparable range of pathogens (Pardeshi, 2020) .

Treatment for UTIs caused by MDR bacteria has gotten more challenging as various resistance mechanisms have arisen. Additionally, MDR species raise costs. Antimicrobial resistance rates for specific years from various nations have been published, despite the lack of extensive surveillance studies on CAUTIs. The primary antibiotics used to treat catheter-associated urinary tract infections (CAUTIs) have the following resistance ranges: ampicilline (21–63.4%), amoxicilline/clavulonic acid (1.2–9.6%), cefuroxime (1–5.4%), ciprofoxacine (0.5–12.9%), trimethoprimesulfamethoxazole (TMT-SMX) (14–45.4%), phosphomycine (0–2.9%), and nalidixic acid (6.3–32.6%). (Öztürk & Murt, 2020).

Treatment for UTI

The use of antibiotics is largely taken into consideration as one of the most advised therapies for UTI. For complex UTIs, EAU guidelines from 2018 propose antimicrobial therapy in addition to amoxicillin plus aminoglycoside and second-generation cephalosporin plus aminoglycoside. Empirical intravenous treatment with a third-generation cephalosporin is advised for complex UTIs with systemic symptoms. According to the 2018 EAU guidelines, antimicrobials such as nitrofurantoin, fosfomycin, cephalexin, or cefaclor can be administered continuously at low doses for three to six months in order to avoid recurrent UTIs (Rodriguez-Mañas, 2020)**.**

Pathogens causing UTI are multiple drugs resistant. Their increase in the resistance towards multiple drugs have raised concern(Belete & Saravanan, 2020). Antibiotics kill or stop the growth of susceptible bacteria, while non-resistant strains can continue to outspread and be transmitted to new hosts. Wild type strains compete with resistant strains in untreated individuals, acting to prevent extensive spread of the resistance. As the pathogens causing UTI require a protein FimH for the adhesion of uropathogens, the antiadhesive strategy of treatment can be an effective aid (Asadi et al., 2019). Antiadhesive property of bioactive compounds can inhibit the interaction of the protein with the urinary tract can be an alternative for treating UTI(Sarshar et al., 2020).

***Vaccinium macrocarpon* (Cranberry)**

Because it contains flavonoids and phenols, *Vaccinium macrocarpon* (cranberry) juice is currently utilized as a traditional medicine. Clinical evidence supports the use of *V. macrocarpon* as a partial or complete substitute for antibiotics. According to studies, cranberry's efficacy varies from person to person and case by case. When used in conjunction with other forms of treatment, *V. macrocarpon* juice can be more beneficial than when taken alone (de Llano et al., 2020). It has been partially effective on recurrent infections where cranberry juice has reduced risks (Havranova et al., 2020) (Jeitler et al., 2022)**.** *V. macrocarpon* extract in combination with D-mannose has been effective in several cases where pathogens were resistant to antibiotics (Rădulescu et al., 2020)

***Garcinia indica* (Kokum)**

The fruit is used in industry for a variety of products, such as pickles, butter, wine, and health beverages. Several phytochemicals of *Garcinia indica (G. indica)* have been studied for their pharmacological effect, including cyanidin-3 glucoside, hydroxycitric acid, garcinol, and cyanidin-3 sambubioside. Anti-obesity, anti-inflammatory, hepatoprotective, antioxidant, anti-arthritic, antibacterial, cardioprotective, antidepressant, and anxiolytic properties of *G. indica* have been demonstrated by the investigations. (Lim et al., 2021) (S. Desai et al., 2022).

Studies on antibacterial property of extracts of *G. indica* have revealed that the extracts of the fruit has the ability to inhibit bacterial growth. Methanol and ethanol extracts have shown zones of inhibition at very low concentration of 500µg/ml (Tharachand et al., 2015). Silver nanoparticles synthesized from the fruit juice of *G. indica* have shown their ability to inhibit

the growth of several bacteria including *E. coli*, *B. subtilis*, *S. aureus* at a minimum inhibitory concentration of 20 mg/ml and 40 mg/ml for *P. aerugenosa* (Sangaonkar & Pawar, 2018). Several studies have reported various benefits of *G. indica* along with their antimicrobial and antioxidant properties. And the studies reveal that the bioactive compounds of *G. indica* have made it a fruit for future therapeutics (Khapare et al., 2020)

Garcinol as antimicrobial and anti-inflammatory compound

Garcinol being one of the major benzophenone present in *Garcinia species* including *G. indica*, has potential as an antibiotic, anticancer, antioxidant and anti-inflammatory effects. As an anti-cancerous agent it has suppressed cancer cell growth in Lung cancer, colorectal cancer, breast cancer, prostate cancer, pancreatic cancer, oral squamous cell carcinoma, cervical cancer and miscellaneous cancer. As an antibiotic it has shown promising activity against *influenza*, *Bacillus anthracis*, *Candida albicans* and *Toxoplasma gondii*. It has also been a very good antioxidant and anti-inflammatory compound with negligible genotoxic effect (Schobert & Biersack, 2019). Garcinol has exhibited its antimicrobial property against several microbes when combined with nanoparticles and has a very low MIC of 62.5µg/ml (Fernando et al., 2019)

Research gap

There are no evidences or studies that highlight the activity of the extracts of *G. indica* specifically against UTI causing pathogens even though the secondary metabolites of the fruit are having potential as antimicrobial and anti-inflammatory agents. They also have ability to inhibit biofilm formation and have broad spectrum activity against various microbes. This creates a scope for research where in the antimicrobial and anti-inflammatory property of the fruit can be studied specifically against uropathogens.

Objectives

To characterize uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* from hospital settings

To test antiadhesive property of potential bioactive molecules against FimH using in-silico studies

To fractionate the phenolic compounds from *Garcinia indica* and *Vaccinium macrocarpon* extracts

To evaluate the antimicrobial activity of the extracts and separated fractions against uropathogenic bacteria

To study the effect of crude and purified extract against test organism using murine model of Urinary Tract Infection

Review of literature

Studies indicate that UTIs are among the most prevalent bacterial infections. However, there is a wide range of clinical presentations of UTIs, from simple, moderate infections to complicated UTIs (cUTIs), pyelonephritis, and severe urosepsis. Stratifying UTI patients is therefore essential. There are many classification techniques for the description and categorization of UTIs, all based on the common rationale that cUTIs are more likely than simple UTIs to recur, chronify, progress, or have a negative result. (Wagenlehner et al., 2020)

Nearly half of women may experience another episode within six to twelve months, and up to 50% will experience the illness at some point in their life. Menopause predisposes women to recurrent UTIs (rUTI) because of changes in the urogenital epithelium and, subsequently, the urogenital microbiota brought on by generally decreased estrogen levels. The recently discovered urobiome is now understood to have a different composition in healthy and sick bladders, contribute to the pathophysiology of rUTI, and may serve as a therapeutic target for the prevention and treatment of rUTI (Jung & Brubaker, 2019).

Women 65 and older are at a much higher risk of infection than other age groups. For younger women, increased sexual activity might be a risk factor. Pregnant women are especially susceptible to the sickness. Pregnant women who have untreated infections run the risk of harming their unborn child. The infection is more common between weeks 22 and 24 of pregnancy, although it can begin as early as the sixth week. The disease may be brought on by the structure of the tiny urethra or even by the fecal flora. Other risk factors include weekly sexual activity, poor hygiene, higher age parity, past infections, and insufficient prenatal care. Elevated sugar and amino acid levels might potentially lead to an illness. (Ranjan et al., 2017), (Medina & Castillo-Pino, 2019)

Type 1 fimbriae are encoded by the majority of *E. coli* strains. The bacterial adhesins, which enable receptor-specific interactions with target cells or tissues, are among the most significant proteins of type 1 fimbriae. The sticky subunit, the mannose-binding protein FimH (30 kDa), is found at the fimbrial tip, whereas the major pilin subunit FimA (15 kDa) makes up the fimbrial body. As a result, pathogens use type 1 fimbrial proteins to connect to the urinary system (Fadhil, 2022)**.** Numerous studies show that the FimH protein promotes the pathogen's adherence to the urinary system, facilitating invasion and colonization. It also results in dysuria and urinary tract discomfort. (Moubayed et al., 2023)

Antibiotic resistance is one of the most serious health problems the world is currently experiencing because there are less effective antibacterial medications accessible. One tactic that has been studied to reduce the development of antibiotic resistance is the use of antiadhesion. Using anti-adhesion medications is a crucial tactic for either treating or avoiding bacterial infections. These medications focus on the virulence and pathogenicity traits of bacteria, such as adhesion and colonization. These medications are believed to be a perfect alternative to antibiotics since they can reduce damage by blocking the infectious process in its early stages. These compounds stop bacteria from attaching to the surface of the host cell by disrupting adhesion biosynthesis, bacterial-host cell assembly, or host receptor assembly. By using an anti-adhesion technique to lower antibiotic resistance or lessen the need for effective antibiotic dosages, bacterial adhesion antibodies can block surface epitopes necessary for bacterial-host cell attachment. (Madle et al., 2018)

Although many researches have documented the anti-uropathogenic and bactericidal activity of various plant extracts, there are few publications on the precise action of the phytoconstituents against uropathogens. Only first antibacterial investigations employing basic methods like disk diffusion, agar well diffusion, or minimum inhibitory concentration (MIC) of the unrefined plant extracts are included in these papers. The best studied natural remedy for urinary tract infections is cranberry, or *V. macrocarpon* Aiton. Some evidence suggests that the proanthocyanins in cranberries prevent pathogens from sticking to the walls of the urinary tract, halting the development of uropathogenesis. Probiotics like Lactobacillus and Bifidobacterium are beneficial microorganisms that may prevent infections in the urogenital tract by using the competitive exclusion principle. There have been few studies on potential vaccination agents and antibodies that target the different toxins and effecter proteins, with the exception of uropathogenic *E. coli*.. (Das, 2020)

The Clusiaceae family includes the little-known fruit tree in the Western Ghats, *G. indica* Choisy (Kokum). Numerous bioactive compounds, such as phenolic acids, flavonoids, citric acids, and others, have been related to the health benefits of *G. indica*. The primary bioactive ingredients are hydroxycitric acid, garcinol, and anthocyanins (cyanidin-3-glucoside and cyanidin-3-sambubioside). Numerous medicinal uses for a range of illnesses, including cancer, inflammation, diabetes, obesity, cardiovascular disease, and neurological problems, have been discovered for the fruit and rinds of *G. indica* (Desai et al. 2022).

Murine models are one of the best ways to research urinary tract infections because of the similarities between human and mouse host defence and particular signalling pathways. Early urinary tract infection triggers the release of interleukins (IL-1b, IL-6, IL-8, IL-10, CCL2, CCL5, and CXC) in both humans and animals. When UTI was created, there was a high frequency of similar host reactions in the mouse and human models. Analysis of the transcriptomes of mice and humans has also revealed a high correlation. There are also many parallels between genes that are activated and down regulated during infection. The innate immune response of mice and humans differs very little, but one significant difference is that during an infection, Toll-like receptor 11 is released in mice as a defence, but not in humans. (Frick-Cheng et al., 2020)

Interleukin-6 (IL-6) is one cytokine that regulates immunity and inflammation. Elevated IL-6 levels are linked to both pyelonephritis and UTIs. Apart from drawing in immune cells, IL-6 starts a chain of genes that produces antimicrobial peptides. Studies have shown that the level of IL-6 in serum can be utilized to distinguish between cystitis and urethritis and to diagnose UTIs. The infected group had higher levels of IL-6 and IL-8 than the control group. IL-6 is secreted by both the infection and renal scarring. This proves that IL-6 is a valuable biomarker for the identification of UTIs (Horváth et al., 2020)

C reactive proteins (CRP), one of the several cytokines produced during inflammation, have been extensively studied as an inflammatory biomarker. CRP levels were elevated in patients with UTIs, however they decrease when antibiotics are taken. CRP can be used as a marker to monitor UTIs because it increases during an infection and decreases as it worsens. This protein is increased during infection, even in infants and young children. (Ashkenazi-Hoffnung et al., 2021)

Objective 1 – To characterize uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* from hospital settings

Introduction

To comprehend UTIs and create efficient treatments, uropathogens must be isolated and characterized from clinical samples. Bacterial colonies are identified and grown for additional investigation using traditional culture-based methods, such as streaking urine samples onto agar substrate. In order to help identify infectious disorders, biochemical assays such as IMVIC offer information on uropathogen virulence, including Gram type and biofilm formation (Karah et al., 2020). By overcoming the drawbacks of culture-based approaches, such as their dependence on living microorganisms and their incapacity to detect uropathogens by focusing on conserved bacterial genome sections, molecular techniques have improved uropathogen identification. Understanding the urine microbiome has been transformed by metagenomic techniques, such as next-generation sequencing (NGS), which reveal low-abundance or unculturable pathogens and offer a thorough picture of microbial diversity. Our knowledge of the etiology of UTIs is improved by these techniques, which also improve detection sensitivity and specificity while providing insightful information about the dynamics and variety of urinary tract microorganisms (Besser et al., 2018).

Studies have identified key virulence factors in uropathogens, such as adhesins, toxins and iron acquisition systems, which facilitate host cell attachment, immune evasion and nutrient acquisition. Molecular techniques like genotyping and gene expression analysis have been instrumental in understanding these mechanisms. The rise of antibiotic resistance among uropathogens highlights the importance of combining genotypic analysis of resistance genes with antimicrobial susceptibility testing to predict susceptibility profiles. This approach aids in guiding appropriate antibiotic use, reducing treatment failures, and limiting the spread of multidrug-resistant uropathogens (Schembri et al., 2001), (Mazzariol et al., 2017).

Methodology

Serial dilution, inoculation and colony counting

Benaka Hospital at Ujire, Belthangady Taluk, Dakshina Kannada District, Karnataka State, provided the sub-cultures of the uropathogens. 5 subcultures were named as SC-1, SC-2, SC-3, SC-4 and SC-5. The sub-cultured pathogens were inoculated to fresh nutrient broth and cultured at 37°C for 16 hours. To obtain 10^1, 10^2, 10^3, and 10^7 dilutions, the original

overnight culture was serially diluted with sterile saline water (0.85%). The agar plates were inoculated with 0.1 ml of each of the diluted samples. The plates with the ideal colony count were taken into consideration for more research, whereas the plates with TNTC and TLTC colonies were disregarded. Dilutions were made in accordance with the stock's CFU/ml to achieve 10^5 CFU/ml. (Coulthard et al., 2010; Mollick et al., 2016; Raksha et al., 2003).

Biochemical characterization of cultured pathogens

Biochemical characterization of the uropathogens were done based on the previous studies of (Dash et al., 2012), (Madeeha Mehboob & Malik Nawaz Shuja, 2021), (Kumar et al., 2017).

To determine the Gram's nature of the pathogens, Gram's staining was used, and the IMViC test was used to describe the cultured pathogens and determine whether they were Enterobacteriacea.

Indole test – 2 ml of freshly cultured media was taken a sterile test tube and Kovac's reagent was added. Test was carried out in accordance with (Kovacs 1928) standard procedure.

MR-VP test –0.7 gm% peptone mixture, 0.5 gm% potassium phosphate, and 0.5 gm% dextrose was used as culture media additives for the MR and VP experiments. For the tests, the indicators were 0.4% methyl red solution, 5% alpha napthanol in absolute alcohol, and 40% sodium hydroxide solution. Methyl red test was carried out in accordance with (Clark, W. M., & Lubs, H. A., 1915) standard procedure and VP test was carried out in accordance with (Voges, O., & Proskauer, B., 1898) standard protocol

Citrate Utilization test - Using 0.1 gm% ammonium di-hydrogen phosphate, 0.5 gm% NaCl, 0.02 gm% $MgSO_4$, 0.008 gm% bromothymol blue, 0.1 gm% di-potassium phosphate, 0.2 gm% sodium citrate, and 1.5 gm% bacterial agar, the citrate utilization test was carried out in accordance with (Simmons J., 1926)'s standard procedure.

Urease Test – (The Christensen, W. B. 1946) standard procedure was followed while performing the urease test on the bacterial isolates. Agar 1.5%, urea 2%, Nacl 0.5 %, KH_2PO_4 0.2 %, and phenol red 0.0012 gm% were used to make the media in a slant orientation.

Molecular identification of the pathogens by 16S RNA Sequencing method

The Genomic DNA of the bacterial strains was extracted by CTAB method (Murray & Thompson, 1980). 16S RNA gene sequencing (Woese & Fox, 1977) was followed for the characterization of the pathogens. The protocol for the isolation and characterization of DNA was based on the experimental studies carried out by (Mazziotti et al., 2018) with slight modification. The DNA obtained was quantified. The DNA was amplified and the amplified fragments of 1200 bp were compared to 100 bp ladder to estimate the quality of the PCR product. By using 27F and 1492R primer DNA was sequenced by Sanger's sequencing method. Sequencing files were obtained and viewed by using ChromasLite software. Sequence data was analysed using BLAST server.

Results

Among the 5 subcultures, the biochemical tests revealed the similarities of 3 sub-cultured pathogens to the characters of the microbes belonging to enterobacteriaceae family wherein 3 subcultures were positive to indole, MRVP, catalase and urease tests. Remaining 2 subcultures were not similar to that of the microbes belonging to enterobacteriacea family. 16S rRNA sequencing and analysis revealed that the 3 subcultures that resembled the characters of enterobacteriaceae family were urpopathogenic *E. coli* and *K. pneumoniae.* subculture 1 (SC-1) and subculture 3 (SC – 3) were uropathogenic *E. coli* and subculture 2 was *K. pneumoniae.*

Discussion

IMViC being the most common tests used to describe the pathogens metabolically (Karah et al., 2020) it was evident that the metabolic pathways of the three sub-cultured pathogens were similar to that of uropathogens. Indole, catalase and urease production by the pathogens confirmed their ability to form biofilm, to colonize and to cause inflammation (Boya et al., 2022), (White et al., 2021), (Lin & Liaw, 2020) and 16S RNA sequencing method being the most suitable method for identifying the bacterial species (Khan et al., 2023), revealed the genetic similarities of the subcultures to uropathogens. Biochemical tests align with the molecular characterization. Both the pathogens being predominant in causing UTI were characterized and they were used for further studies to determine the activity of test samples on them.

Objective 2 – To test antiadhesive property of potential bioactive molecules against FimH using in-silico studies

Introduction

Since molecular docking is a crucial component of computer-aided drug design (CADD), recent developments in the field have made it easier for researchers to screen many compounds quickly. In order to determine the ligand's affinity for binding the protein, it involves analyzing the protein-ligand interaction and computing the binding energy. The goal of molecular docking simulations is to understand the complex atomic-level dance between ligands and proteins. The understanding of the complimentary nature of molecular structures, which is embodied in Emil's lock-and-key model, is essential to this endeavor. (Fischer et al., 2021), (Dhakal et al., 2022).

As type 1 fimbriae are important for the uropathogen to get adhered to the urinary tract, *FimH* protein present at the tip of the fimbriae ensures adhesions by interacting with the high-mannosylated uroplakins and further leading to the colonization of the bacteria (Sarshar et al., 2020). The study compares the affinity of various secondary metabolites of *G. indica* (Kokum) and *V. macrocarpon* (Cranberry) towards the protein *FimH*. The compounds having greater affinity can be a very good antiadhesin that can inhibit the adhesion of uropathogens to the urinary tract.

Methodology

The tools used for docking and visualization and the protocol used for conducting molecular docking simulation was based on the studies of (Prashanth et al., 2021), (Valdés-Tresanco et al., 2020) and (Di et al., 2017). Review of literature assisted the selection of secondary metabolites for studying their anti-adhesive nature. Secondary metabolites from *V. macrocarpon* were chosen based on the studies of (Scharf et al., 2020) and the secondary metabolites from *G. indica* were chosen based on the studies of (Garden, 2016; Jagtap et al., 2015; Murthy et al., 2019).

The metabolites showing greater binding affinity among the chosen potential candidates from *V. macrocarpon* and *G. indica* were identified and the affinity was compared for better understanding.

Molecular docking study was conducted using AutoDock Vina 1.1.2 to get a better understanding of the complex interaction and the conformational changes that takes place

during complex formation within the binding cavity of protein. The pre-docking preparation was done based on Autodock4.2 protocol using Autodock Tools 1.5.6

Using GROMACS-2018.1, molecular dynamics simulations were used to examine the stability of the docked complex in order to comprehend its behavior and conformational changes at the structural level. The CHARMM27 force field parameter was used to run the MD simulation for 100 ns. The SwissParam service (https://www.swissparam.ch/) was utilized for ligand topology, whereas the gmx tool was used to build protein topology files. The trajectories were then shown for Radius of Gyration (Rg), Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), SASA (Solvent Accessible Surface Area), and hydrogen bond using the XMGRACE software.

To understand the energy formed during protein-ligand complex formation after the dynamic study, molecular mechanics/Poisson-Boltzmann Surface Area (MM-PBSA) technique was employed. The result obtained after the simulation was considered to calculate free-binding energy by using gmmpbsa programme.

MD modeling was used to confirm the docked structure of the kaempferol-bound FimH complex and the garcinol-bound FimH complex. Plotting the graph for RMSD, RMSF, Rg, SASA, and H-bond allowed for the analysis of the result. MD modeling was utilized in order to confirm the docked garcinol bound FimH complex. The purpose of the simulation was to better understand the stability of the complex produced during the docking process and the structural rearrangement of the garcinol-FimH complex.

Results

Among all the screened secondary metabolites from *G. indica* and *V. macrocarpon*, a benzophenone present in *G. indica* and a flavonol present in *V. macrocarpon* showed better activity. Among the two garcinol had higher affinity in binding the adhesive protein *FimH.* Garcinol had a binding energy of -8.5 kcal/mol whereas kaempferol had -7.9 kcal/mol. Garcinol showed 3 hydrogen bonds among 12 interactions whereas kaempferol showed 3 hydrogen bonds among 7 interactions. RMSD plot revealed that the garcinol complex and protein is within the range 0f 0.2-0.25 nm, whereas kaempferol complex is within the range of 0.3-0.35 nm. RMSF, Rg and SASA analysis shows the stability of the docked complex where there were fewer fluctuations.

Discussion

Surface area (SASA), radius of gyration (Rg), Root Mean Square Fluctuation (RMSF), and Root Mean Square Deviation (RMSD) being the parameters that helps to estimate the stability of the protein ligand interaction (Islam et al., 2021), were considered for the study that exhibits the ability of garcinol to bind *FimH* efficiently. The MM-PBSA findings suggest that garcinol's stronger binding is driven by its ability to form a network of interactions that stabilize the complex, which kaempferol lacks (Tuccinardi, 2021). Kaempferol has been studied extensively for its nephroprotective effect (Alkandahri et al., 2024) and also it is one of the polyphenols from Cranberry that has been considered as an antiadhesin against uropathogens (Yu & Shi, 2021). The results of the study reveal the potential of garcinol to be an antiadhesin is equally promising to kaempferol.

Objective 3 – To fractionate the phenolic compounds from *Garcinia indica* and *Vaccinium macrocarpon* extracts

Introduction

Alkaloids, flavonoids, terpenoids, phenolic acids, and essential oils are among the complex mixture of bioactive components found in herbal extracts, which are produced using a variety of extraction techniques such as maceration, decoction, and distillation. These phytochemicals are useful tools for drug discovery and development because of their wide range of pharmacological properties, which include antibacterial, anti-inflammatory, antioxidant, and immunomodulatory actions. Herbs are regarded as safe and efficient antibacterial agents. Additionally, they can work more effectively in tandem with antibiotics to reduce the likelihood of developing resistance to the combination of herbal medications. (Liang et al., 2022)

The effectiveness of the extraction is influenced by a number of factors, including the target compound's physical and chemical characteristics, extraction procedures, the optimal use of solvents, physiological conditions during the extraction, etc. Some of the earliest extraction techniques that include heating the solvent include Soxhlet extraction, maceration, distillation, and others. The effectiveness of the extraction process is also being improved by the employment of contemporary methods such as pressurized liquid extraction (PLE), high intensity pulsed electric field (HIPEF), enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), etc.

Identification of the appropriate bioactive compounds, as well as the separation and purification of the secondary metabolites, have been made easier by the use of extraction techniques in conjunction with analytical techniques such as chromatography (High Performance Liquid Chromatography, Thin Layer Chromatography, Column Chromatography, etc.), NMR, UV-Vis spectroscopy, etc. These methods allow plants and their phytochemicals to be used as efficiently as possible and can be included into the development of new drugs to combat a variety of diseases. (Ivanović et al., 2020)

Methodology

Preparation of Herbal extracts

Dried fruit rinds of *G. indica* was obtained from the growers. 20 grams of the dried rinds were transferred to the Soxhlet apparatus (Soxhlet et al., 1879) and extracted with methanol

for 9 hours (D. Desai et al., 2019). The extract thus obtained was concentrated using rotor evaporator to get a yield of 3.9 grams. The step was repeated to get crude methanolic extract. To get an aqueous extract, 20 grams of dried fruit rinds of *G. indica* was suspended in 300 ml of distilled water in 5 batches. The extract was kept on a water bath to get a concentrated crude extract. (Deore et al., 2011), (Tharachand et al., 2015).

V. macrocarpon (Cranberry) extract was obtained from the capsules that are available as a drug against UTI.

Aqueous and methanol extract of *G. indica* and the *V. macrocarpon* extract was subjected to Liquid Chromatography and Mass spectroscopy. Waters (USA) LCMS instrument was used for the study where the instrument was equipped with a coloumn BEH C18, 50 x 1.0, 1.7u. It had a photodiode array detector. Gradient elution was used with a 0.1% formic acid in water as solvent A and acetonitrile as solvent B.

Purification of the phenols

Purification of garcinol from *G. indica* crude extract: The crude extract was re-dissolved in ethyl acetate. The latter was washed with water. The concentrated ethyl acetate extract was subjected to column chromatography using ethyl acetate/hexane mixture at 1:2 ratio. Yellow fraction was collected and concentrated with hexane to obtain garcinol (Schobert & Biersack, 2019). The fraction obtained was subjected to LCMS with positive electron spray ionization.

Purification of kaempferol from Cranberry extract: The cranberry extract was redissolved in methanol and hydrolysed by reflux in HCL (2M). The reaction mixture was cooled with ice water and subsequently extracted with ethyl acetate. Solvent was evaporated and then washed with water and then with saturated Nacl solution. Coloumn chromatography was performed by using silica gel as a stationary phase and chloroform: acetone: formic acid (75: 16.5: 8.5 v/v/v) as mobile phase (Zeka et al., 2015). Fraction thus obtained was subjected to LCMS with negative electron spray ionization.

The extracts and purified compounds were subjected to Liquid Chromatography and Mass Spectroscopy (LC-MS) for identification of the compounds and to check the purity of the purified phenols.

Results

The peaks in the LCMS graph revealed the presence of garcinol, hydroxy citric acid, cyanidin-3-glucoside, cyanidin-3-sambubioside, xanthochymol and other class of compounds in the extracts of *G. indica* and presence of apigenin, quercitine, kaempferol and other compunds in *V. macrocarpon* extract. After purification the liquid chromatography revealed that the garcinol was 97% pure whereas kaempferol was partially pure with the purity of 50%.

Discussion

Methanol and water are the solvents that can be utilized for the extraction of polyphenols due to their polarity. Soxhlet extraction of herbs with these solvents can extract polyphenols from various herbs (Alara et al., 2021). Electrospray Ionization (ESI) contributes to the accuracy of detection of various compounds including thermally labile, volatile and polar compounds. Due to its high sensitivity and accuracy LC-MS has been widely used in analysis of broad spectrum of compounds (Beccaria & Cabooter, 2020). Studies of (Denis et al., 2020) confirms the mass and retention times of the detected compounds. The peaks revealed the purity of garcinol was 97% compared to that of kaempferol was 50% (Bhagwat & Datar, 2014; Bharate et al., 2014; Rao Gajula et al., 2023). This explains why the *G. indica* extract included a variety of phenols, including cyanidin-3-sambubioside, xanthochymol, isogarcinol, hydroxycitric acid, and garcinol. The effectiveness of the extraction and purifying process is demonstrated by the purity of garcinol. Kaempferol was less pure than garcinol, which might have been caused by its extraction from the capsule. The metabolites that are so separated can be used for additional research on their antibacterial and antiadhesive qualities.

Objective – 4: To evaluate the antimicrobial activity of the extracts and separated fractions against uropathogenic bacteria

Introduction

Herbal extracts are proving to be having a promising effect against pathogens causing urinary tract infection. Less toxic secondary metabolites having minimum side effects are the future to treat UTI that can be an efficient replacement to the existing antibiotics and also they can overcome drug resistance of pathogens. As the plants have multiple compounds, there are multiple mechanism of the herbal extract against pathogens that make the pathogens difficult to build resistance against the drug. Herbal extracts activity are more efficient when they are administered crude rather than administering the purified compound as they have synergistic effect (Allami et al., 2020).

Among the available medicines *V. macrocarpon* (Cranberry) extract is a herbal medicine that is being prescribed against urinary tract infection. Proanthocyanidins, flavonols, polyphenols and other secondary metabolites from the fruit is said to be having the anti-adhesive properties that prevent the attachment of uropathogenic bacteria, such as *E. coli*, to the uroepithelial cells lining the urinary tract. Proanthocyanidins present in cranberry extract interfere with the bacterial fimbriae responsible for adhesion, thereby inhibiting bacterial colonization and subsequent infection. Additionally, cranberry extract may exert anti-inflammatory effects and promote urinary tract health through its antioxidant properties, further contributing to its therapeutic efficacy against UTIs (Gbinigie et al., 2021).

The extracts of *G. indica* also have antimicrobial activity against several classes of microbes including *Enterococcus faecalis, Streptococcus mutans, E. coli, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium etc.* This ability of the fruit to inhibit the growth of several pathogens are mainly due to the presence of the secondary metabolites like Garcinol, Hydroxycitric acid, cyanidin-3-glucoside, cyaniding-3-sambubiosdie etc. that exhibits antimicrobial property (Rai & Sayeed, 2022; Sushma et al., 2020). The study concentrates on the antimicrobial activity of *G. indica* extracts against sub-cultured urinary tract infection causing pathogens.

Methodology

Agar well diffusion method

To evaluate the antimicrobial property of the herbal extracts Kirby Bauer's disc diffusion (Aw, B. 1966) standard protocol and Vincent's agar well diffusion method (Vincent et al., 1944) standard protocol was followed. The subcultures were inoculated to fresh nutrient broth and 24 hours old culture was used for further studies. To the sterile agar plates 100µl ($1x10^5$ CFU/ml) of the inoculum was transferred and spread. A well was made using a cork borer and 100 µl of the herbal extract and the purified compounds of desired concentration were poured into the well and the plates were incubated at 37^oC. The zone of inhibition formed by the extracts were observed, measured, and recorded. Equal volume of sterile distilled water and methanol was poured and were considered as negative control. Antibiotics discs having Ampicillin (10 mcg), Ciprofloxacin (30 mcg), Cephalothin (30 mcg), Trimoxazole (25 mcg), Gentamicin (10 mcg), Nalidixic acid (30 mcg), Nitrofurantoin (300 mcg) and Norfloxacin (10 mcg) and *V. macrocarpon* extract obtained from the capsules were used as positive control. (Tabassum et al., 2013), (Komal et al., 2018)

Results

G. indica aqueous extracts and methanol extracts exhibited MIC of 5mg/ml. *V. macrocarpon* extract which was used as positive control showed MIC of 8mg/ml. Purified showed MIC of 1.5mg/ml and kaempferol's MIC was 2mg/ml. Among the 8 antibiotics used as positive control the pathogens were resistant to 50% of the antibiotics. Both the pathogens were highly sensitive to ciprofloxacin.

Discussion

Purified garcinol and *G. indica* extracts were able to stop uropathogens from growing. The test samples and the *V. macrocarpon* extracts had the same potency. Both extracts' MICs were marginally elevated, which could be because several chemicals were involved. According to studies, a herbal extract's antibacterial properties may result from the combined action of several different components rather than from a single ingredient. The infections' resistance indicates that they are resistant to several drugs, and the pattern of resistance varies depending on the location. (Petca et al., 2020).

For plant extract to work, the MIC should be less than 2 mg/ml. The amount of secondary metabolites in the herbal extracts is responsible for a large portion of their antibacterial

activity. Since the extract contains a variety of chemicals, not all of them may be involved in the antibacterial activity. Therefore, the herbal extract's MIC is typically a little higher. According to studies, taking antibiotics without any phytochemicals would reduce their effectiveness, while taking herbal extracts along increases the action of antibiotics. It might be the result of phytochemicals and antibiotics working in concert. Particularly, polyphenols that contribute to the infections' cell membrane rupture can work better in combination with antibiotics. (Álvarez-Martínez et al., 2021)

The presence of many kinds of secondary metabolites, particularly polyphenols, may be the reason why both *G. indica* and *V. macrocarpon* have demonstrated their capacity to suppress the proliferation of uropathogens. Alkaloids can prevent infections from dividing, phenols and tannins can react with microbial proteins, flavonoids in plants can bind with bacterial cell walls and proteins, and so on (Hemeg et al., 2020). Studies have revealed that polyphenols and proanthocyanidins from *V. macrocarpon* have great antiadhesive property that can inhibit the adhesion of uropathogens (de Llano et al., 2020)

Objective 5 - To study the effect of crude and purified extract against test organism using murine model of Urinary Tract Infection

Introduction

Herbal extracts' in vitro antibacterial activity must be confirmed through in vivo testing. Animal models are the most effective way to assess the pharmacodynamic/pharmacokinetic connection of herbal extracts or any antibacterial drug. Numerous features of UTIs, including as bacterial colonization, host immune responses, tissue damage, and treatment outcomes, can be simulated and investigated using animal models, which offer a controlled experimental platform. Researchers can learn more about the dynamics of infection propagation, the function of particular virulence factors, and the effectiveness of new antimicrobial medicines by using animal models (Chockalingam et al., 2019).

Drug action against bacteria that cause UTIs is typically studied using mouse/rat models. Urinary tract infections in animal models are typically caused by catheterization, which introduces germs into the bladder. For this, a microbial inoculum containing 10^7 CFU/ml is employed. Using samples of the bladder, kidney, or even blood allows the researcher to examine how the medication affects the infection, which aids in determining dosage and evaluating the medication's antimicrobial activity. (Zhao et al., 2016).

Methodology

Animal studies were conducted based on the standard protocol, guidelines, and approved study proposal by the ethical committee with the study number RR231131-1134/PC/UTI/07-2023. Strain of the animal used for the study was *Swiss albino* female mice of 8-10 weeks of age. Body weight of the animals used for the study was 20-25 grams.

Animal experiment was conducted in accordance with the guidelines of the committee for the control and supervision of the experiments on animals (CCSEA registration number – 1803/PO/RcBi/S/2015/CCSEA).

Swiss albino mice were divided into 8 groups each group having 6 mice. Group 1 was considered as negative control group, Group 2 was pathological control induced with bacteria without treatment, Group 3 was positive control treated with ciprofloxacin 50 mg/Kg, Group 4 was treated with *V. macrocarpon* 250 mg/kg, Group 5 was treated with *G. indica* methanol extract 250 mg/kg, Group 6 was treated with *G. indica* water extract 250 mg/kg, Group 7 was

treated with partially purified garcinol 250 mg/kg and Group 8 was treated with *G. indica* water extract 250 mg/kg and ciprofloxacin 50 mg/kg for 15 days. Dosage of the herbal extracts determined for the experiments were based on the suggestions of the study (Heinrich et al., 2020; Nair & Jacob, 2016)

Urine catheters were prepared for inoculation, inoculum of 50µl having CFU of 10^7CFU/ml concentration were drawn and injected to the bladder of the mice and infection was confirmed by collecting the urine sample and re-inoculating on agar plates and counting the colonies. After infection, herbal extracts and antibiotics were administered according to the group description. pH of the urine, renal testing, crp and IL-6 were the parameters to test the rate of infection and efficacy of the herbal drugs on the infection. They were tested by extracting the serum of the test organism. The protocol followed for the infection and urine analysis were according to the previous studies of (Hung et al., 2009) (Conover et al., 2015), (Arifianto et al., 2020; Ching et al., 2018; Hannan & Hunstad, 2016; Li et al., 2019; Shi et al., 2023; Vachvanichsanong, 2021).

At the end of the treatment period (Day 15) for all groups, all the animals were sacrificed by using isoflurane and subjected to gross pathological examination. Urinary bladder was collected for further histopathological studies

Results

Considering the variation in the pH level of the experimental groups, the urine pH was tested at day 0, 7 and 14 of the experiment. The experimental group considered as pathological control had pH of 5.5 – 6.4 in day 0, 6.8 -7.7 in day 7 and 8 – 8.8 in day 14. Considering the IL-6 and CRP that were the biomarkers to test infection levels, pathological control showed the IL-6 concentration of 20.70 ± 2.67 (pg/ml) and CRP of 8.42 ± 0.12 (mg/dL). Considering the Renal functions, concentration of urea, creatinine, sodium and potassium in the serum of pathological control was 53.90 ± 1.10 (mg/dL), 1.77 ± 0.03 (mg/dL), 127.33 ± 0.99 (mmol/L) and 2.77 ± 0.08 (mmol/L) respectively. Infection altered the urine pH which increased as the infection increased. The concentration of the biomarkers revealed that higher the level of infection more is their concentration and the infection affects kidney function which was evident in the concentration of urea, creatinine, sodium and potassium.

The effect of the test substances were analysed by the ability of the test samples to control the infection thus controlling the alteration in the variables. Among all the test samples the

herbal extracts showed moderate activity, whereas antibiotic, purified garcinol and combination of herbal extract and antibiotic showed highest activity. Herbal extract and antibiotic was found to be the best formulation that controlled the infection effectively which showed the pH levels of 5.7-6.6 in day 0, 6.2-7.3 in day 7 and 6.1-6.4 in day 14. IL-6 and CRP concentrations were 18.73 ± 2.10 (pg/ml) and 5.68 ± 0.16 (mg/dL) respectively. Concentrations of urea, creatinine, sodium and potassium were 32.38 ± 0.64 (mg/dL), 0.64 ± 0.07 (mg/dL), 147.00 ± 0.73 (mmol/L) and 4.63 ± 0.05 (mmol/L) respectively.

Discussion

Studies have shown that CRP is another important metric that can be used to gauge the severity of a urinary tract infection. The severity of the infection increases with a higher CRP level in blood serum, and vice versa. Patients with pyelonephritis have greater CRP levels than those with acute lower urinary tract infections (Narayan Swamy et al., 2022). When patients with UTI exhibit elevated levels of urea and creatinine in their urine sample, the levels of these substances can also be used to determine the severity of the infection. Increased urea and creatinine indicate compromised renal function brought on by an illness. (CRISTEA et al., 2019). The pathogens causing UTI can split urinary urea thus increasing the urinary pH (Hsiao et al., 2019). Increased urea and creatinine levels in serum depicts the inability of the kidney to filter them from blood and the malfunction of kidney can be due to infection (Almaiman et al., 2021). The degree of infection can also be determined by changes in the urine's potassium and sodium levels. According to studies, inflammation and renal scarring are associated with hyponatremia, or a drop in the serum sodium level. Antidiuretic hormone secretion may be affected by IL-6 production, and inflammation may result in elevated prostaglandin secretion, which may inhibit salt transport.

Studies also reveal that renal parenchymal involvement in the infection and inflammation can make the organism hyporesponsive to aldosterone that concentrates urine (Pappo et al., 2020). Previous studies on effect of infection on urothelial cells say that urothelial cell mucosa acts as a first line of defence against uropathogens. When the infection is severe the urothelial cells stops proliferating and apoptosis is induced. In some cases lack of urothelial cells have led to recurrent urinary tract infection (Jhang et al., 2021). Chronic infection leads to exfoliation of urothelium from the connective tissue. This increases inflammation, thus increasing the frequency to urinate and polyuria (Zhu et al., 2021).

G. indica extracts have been efficient in controlling the infection which was evident in the results as the experimental group treated with the extract have shown less variation in the urine pH, less concentration of the biomarkers and less concentration of urea, creatinine, sodium and potassium. The activity enhanced when it was combined with antibiotic. This reveals the synergistic effect of herbal extract when combined with the antibiotic. Hence the extracts of *G. indica* and purified garcinol can be considered for further studies as antimicrobial and anti-inflammatory agent against uropathogens.

Statistical Analysis

When there is one variable and multiple groups for analysis, one way ANOVA is the most suitable method followed. One way ANOVA being an improved version of paired t-test helps to analyse experimental data. In the study IL-6 and CRP levels of serum being the important variables, comparing them among test groups helps to understand the level of infection and the ability of the test samples to control the infection. It compares means of multiple groups and assists as a proper statistical tool (Gillard J., 2020). Statistical analysis show that the p value is <0.05 when compared among the test groups which says that null hypothesis can be rejected.

Summary

The hypothesis of the study was the extracts of *G. indica* has antimicrobial property against uropathogens that can inhibit the growth of uropathogens and also the extracts have anti-inflammatory property that can minimize the inflammation caused by the infection. Hence according to the first objective the subcultures obtained from the nearby hospitals were characterized both biochemically and at molecular level. After confirmation of the pathogens being uropathogenic *E. coli* and *K. pneumoniae*, they were considered for further studies. As *G. indica* extracts contain many bioactive compounds, the anti-adhesive potential of the candidates of *G. indica* were tested and compared with the bioactive compounds of *V. macrocarpon*. Garcinol being the candidate having more affinity to bind the protein FimH (Necessary for the adhesion of uropathogen to urinary tract), it was considered for purification and to study antimicrobial property.

Aqueous and methanol were chosen as solvents because of their polarity and the extract was obtained through soxhlet extraction. Garcinol was purified through column chromatography. *V. macrocarpon* extract was obtained from the already available capsules that could be

treated as positive control and kaempferol was partially purified through column chromatography from the *V. macrocarpon* extract. Agar well diffusion method was followed to estimate the antimicrobial property of the extracts and purified phenols. Antimicrobial property of *G. indica* was compared to that of *V. macrocarpon* and garcinol was compared to kaempferol. Antibiotic resistance by the pathogens were also studied by disc diffusion method. Extracts of *G. indica* and purified garcinol was efficient in inhibiting the growth of uropathogens where both the extracts having the MIC of 5mg/ml and purified garcinol having the MIC of 1.5mg/ml.

Anti-inflammatory property of the extracts along with their ability to control inflammation was studied invivo using murine models. IL-6 and CRP were considered as biomarkers for analysing inflammation level, renal functions and urine pH were analysed to understand the functioning of urinary system during the infection. Experimental groups were induced with the infection and the test substances were administered to the experimental groups in order to understand the ability of the test substances to control infection and inflammation. Activity of aqueous and methanol extracts *G. indica* and purified garcinol was tested using *V. macrocarpon* extract and antibiotic as positive control. Synergistic effect of the herbal extract was also tested by combining it with antibiotic. Test results reveal that synergistic effect of the herbal extract was more efficient in controlling the inflammation, purified garcinol also showed considerable effect. Herbal extracts showed moderate activity.

The experimental results reveal the ability of the *G. indica* fruit rind extracts and purified garcinol to be a potential drug candidate against uropathogens. Statistical analysis including One way ANOVA shows the p value <0.05 when all the test groups were compared to each other. Statistical analysis rejects null hypothesis and accepts alterative hypothesis. Considering this for further research, proper outcomes can provide an alternative approach to treat UTI and also an underutilized fruit can be converted as a bio resource.

References

Abou Heidar, N., Degheili, J., Yacoubian, A., & Khauli, R. (2019). Management of urinary tract infection in women: A practical approach for everyday practice. *Urology Annals*, *11*(4), 339–346. https://doi.org/10.4103/UA.UA_104_19

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current Research in Food Science*, *4*(March), 200–214. https://doi.org/10.1016/j.crfs.2021.03.011

Alkandahri, M. Y., Sadino, A., Pamungkas, B. T., Oktoba, Z., Arfania, M., Yuniarsih, N., Wahyuningsih, E. S., Dewi, Y., Winarti, S. A., & Dinita, S. T. (2024). Potential Nephroprotective Effect of Kaempferol: Biosynthesis, Mechanisms of Action, and Clinical Prospects. *Advances in Pharmacological and Pharmaceutical Sciences*, *2024*(1) . https://doi.org/10.1155/2024/8907717

Allami, R. H., Mouhamad, R. S., Abdulateef, S. A., & al-Khafaji, K. abedalelah. (2020). Antimicrobial Activity of Herbal Mixture Extract Combination on Microorganisms Isolated from Urinary Tract infection. *Bionatura*, *5*(4), 1346–1351. https://doi.org/10.21931/RB/2020.05.04.11

Almaiman, L., Allemailem, K. S., El-Kady, A. M., Alrasheed, M., Almatroudi, A., Alekezem, F. S., Elrasheedy, A., Al-Megrin, W. A., Alobaid, H. M., & Elshabrawy, H. A. (2021). Prevalence and significance of pyuria in chronic kidney disease patients in saudi arabia. *Journal of Personalized Medicine*, *11*(9). 831 https://doi.org/10.3390/jpm11090831

Álvarez-Martínez, F. J., Barrajón-Catalán, E., Herranz-López, M., & Micol, V. (2021). Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. *Phytomedicine*, *90*. https://doi.org/10.1016/j.phymed.2021.153626

Arifianto, D., Adji, D., Sutrisno, B., & Rickyawan, N. (2020). Renal Histopathology, Blood Urea Nitrogen and Creatinine Levels of Rats With Unilateral Ureteral Obstruction. *Ijvs*, *1*(1), 1–9. https://doi.org/10.22146/ijvs.46354

Asadi, A., Razavi, S., Talebi, M., & Gholami, M. (2019). A review on anti-adhesion therapies of bacterial diseases. *Infection*, *47*(1), 13–23. https://doi.org/10.1007/s15010-018-1222-5

Ashkenazi-Hoffnung, L., Livni, G., Scheuerman, O., Berger, I., Eden, E., Oved, K., Shani, L., Kronenfeld, G., Simon, E., Boico, O., Navon, R., Gottlieb, T. M., Barash, E., Paz, M., Yuhas, Y., Berent, E., & Ashkenazi, S. (2021). Differential Serum and Urine CRP, IP-10, and TRAIL Levels in Pediatric Urinary Tract Infection. *Frontiers in Pediatrics*, *9*(December), 1–8. https://doi.org/10.3389/fped.2021.771118

Aw, B. (1966). Antibiotic susceptibility testing by a standardized single disk method. A*m J Clin Pathol*, 45, 493-496.

Beccaria, M., & Cabooter, D. (2020). Current developments in LC-MS for pharmaceutical analysis. *Analyst*, *145*(4), 1129–1157. https://doi.org/10.1039/c9an02145k

Belete, M. A., & Saravanan, M. (2020). A systematic review on drug resistant urinary tract infection among pregnant women in developing countries in africa and asia; 2005-2016. *Infection and Drug Resistance*, *13*, 1465–1477. https://doi.org/10.2147/IDR.S250654

Besser, J., Carleton, H. A., Gerner-Smidt, P., Lindsey, R. L., & Trees, E. (2018). Next-generation sequencing technologies and their application to the study and control of bacterial infections. *Clinical Microbiology and Infection*, *24*(4), 335–341. https://doi.org/10.1016/j.cmi.2017.10.013

Bhagwat, M., & Datar, A. (2014). Isolation and Identification of Antibacterial Compounds from the Extracts of Garcinia indica and Curcuma aromatica, using Bioautography and Mass Spectrometric Techniques. *Journal of Biologically Active Products from Nature*, 4(4), 295–302. https://doi.org/10.1080/22311866.2014.961102

Bharate, J. B., Vishwakarma, R. A., Bharate, S. B., Kushwaha, M., & Gupta, A. P. (2014). Quantification of the polyisoprenylated benzophenones garcinol and isogarcinol using multiple reaction monitoring LC/electrospray ionization-MS/MS analysis of ultrasound-assisted extracts of Garcinia indica fruits. *Journal of AOAC International*, *97*(5), 1317–1322. https://doi.org/10.5740/jaoacint.13-137

Boya, B. R., Lee, J. H., & Lee, J. (2022). Antibiofilm and Antimicrobial Activities of Chloroindoles Against Uropathogenic E.coli. *Frontiers in Microbiology*, *13*(June). https://doi.org/10.3389/fmicb.2022.872943

Clark, W. M., & Lubs, H. A. (1915). The differentiation of bacteria of the colonaerogenes family by the use of indicators. *The Journal of Infectious Diseases*, 160-173.

Ching, C. B., Gupta, S., Li, B., Cortado, H., Mayne, N., Jackson, A. R., McHugh, K. M., & Becknell, B. (2018). Interleukin-6/Stat3 signaling has an essential role in the host antimicrobial response to urinary tract infection. *Kidney International*, *93*(6), 1320–1329. https://doi.org/10.1016/j.kint.2017.12.006

Chockalingam, A., Stewart, S., Xu, L., Gandhi, A., Matta, M. K., Patel, V., Sacks, L., & Rouse, R. (2019). Evaluation of immunocompetent urinary tract infected Balb/C mouse model for the study of antibiotic resistance development using Escherichia Coli CFT073 infection. *Antibiotics*, *8*(4). 170. https://doi.org/10.3390/antibiotics8040170

Christensen, W. B. (1946). Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. *Journal of bacteriology*, 52(4), 461-466.

Conover, M. S., Flores-Mireles, A. L., Hibbing, M. E., Dodson, K., & Hultgren, S. J. (2015). Establishment and characterization of UTI and CAUTI in a mouse model. *Journal of Visualized Experiments*, *2015*(100), 1–12. https://doi.org/10.3791/52892

Coulthard, M. G., Kalra, M., Lambert, H. J., Nelson, A., Smith, T., & Perry, J. D. (2010). Redefining urinary tract infections by bacterial colony counts. *Pediatrics*, *125*(2), 335–341. https://doi.org/10.1542/peds.2008-1455

CRISTEA, O. M., AMZOIU, M., ENE, C., AMZOIU, E., POPESCU, F., POPESCU, F.-D., RADU, L., & SIRBU, R. (2019). Biochemical Changes in the Evolution of Chronic Kidney Disease Associated With Urinary Tract Infections. *Journal of Science & Arts*, *19*(2), 425–442.

Das, S. (2020). Natural therapeutics for urinary tract infections—a review. *Future Journal of Pharmaceutical Sciences*, *6*(1). https://doi.org/10.1186/s43094-020-00086-2

Dash, S. K., Chakraorty, S. P., Mandal, D., & Roy, S. (2012). Isolation and Characterization of Multi Drug Resistant Uropathogenic Escherichia Coli From Urine Sample of Urinary Tract Infected Patients. *International Journal of Life Science and Pharma Research*, *2*(1), 25–39.

de Llano, D. G., Moreno-Arribas, M. V., & Bartolomé, B. (2020). Cranberry polyphenols and prevention against urinary tract Infections: Relevant considerations. *Molecules*, *25*(15). https://doi.org/10.3390/molecules25153523

Denis, M. C., Dubé, P., Dudonné, S., Desjardins, Y., Matei, C., Delvin, E., Levy, E., & Furtos, A. (2020). Characterization of bioactive cranberry fractions by mass spectrometry. *Canadian Journal of Chemistry*, *98*(9), 589–596. https://doi.org/10.1139/cjc-2020-0070

Deore, A. B., Sapakal, V. D., Dashputre, N. L., & Naikwade, N. S. (2011). Antiulcer activity of Garcinia indica linn fruit rinds. *Journal of Applied Pharmaceutical Science*, *1*(5), 151–154.

Desai, D., Dhundale, V., Kasar, K., & Desai, D. (2019). *Antibacterial and phytochemical evaluation of various extracts of G . indica (Kokum) leaves and bark*. *7*(4), 207–211.

Desai, S., Sharma, P., Kashyap, P., Choudhary, B., & Kaur, J. (2022). Bioactive compounds, bio-functional properties, and food applications of Garcinia indica: A review. *Journal of Food Biochemistry*, *46*(10), e14344. https://doi.org/10.1111/JFBC.14344

Dhakal, A., McKay, C., Tanner, J. J., & Cheng, J. (2022). Artificial intelligence in the prediction of protein-ligand interactions: recent advances and future directions. *Briefings in Bioinformatics*, *23*(1), 1–23. https://doi.org/10.1093/bib/bbab476

Di Muzio, E., Toti, D., & Polticelli, F. (2017). DockingApp: a user friendly interface for facilitated docking simulations with AutoDock Vina. *Journal of Computer-Aided Molecular Design*, 31, 213-218.https://doi.org/10.1007/s10822-016-0006-1

Fadhil, R. A. (2022). Detection of fimH virulence gene in uropathogenic Escherichia coli Using PCR. *Journal of University of Anbar for Pure Science*, *14*(2), 1–4. https://doi.org/10.37652/juaps.2022.172335

Fernando, H. N., Kumarasinghe, K. G. U. R., Gunasekara, T. D. C. P., Wijekoon, H. P. S. K., Ekanayaka, E. M. A. K., Rajapaksha, S. P., Fernando, S. S. N., & Jayaweera, P. M. (2019). Synthesis, characterization and antimicrobial activity of garcinol capped silver nanoparticles. *Journal of Microbiology and Biotechnology*, *29*(11), 1841–1851. https://doi.org/10.4014/jmb.1904.04032

Fischer, A., Smieško, M., Sellner, M., & Lill, M. A. (2021). Decision Making in Structure-Based Drug Discovery: Visual Inspection of Docking Results. *Journal of Medicinal Chemistry*, *64*(5), 2489–2500. https://doi.org/10.1021/acs.jmedchem.0c02227

Frick-Cheng, A. E., Sintsova, A., Smith, S. N., Krauthammer, M., Eaton, K. A., & Mobley, H. L. T. (2020). The gene expression profile of uropathogenic escherichia coli in women with uncomplicated urinary tract infections is recapitulated in the mouse model. *MBio*, *11*(4), 1–16. https://doi.org/10.1128/mBio.01412-20

Garden, T. B. (2016). Diversity of Garcinia species in the Western Ghats: Phytochemical Perspective. In *Diversity of Garcinia species in the Western Ghats: Phytochemical Perspective* (Issue November). https://doi.org/10.25173/978-81-924674-5-0

Gbinigie, O. A., Spencer, E. A., Heneghan, C. J., Lee, J. J., & Butler, C. C. (2021). Cranberry extract for symptoms of acute, uncomplicated urinary tract infection: A systematic review. *Antibiotics*, *10*(1), 1–14. https://doi.org/10.3390/antibiotics10010012

Gillard, J., (2020). One-way analysis of variance (anova). *A First Course in Statistical Inference*, 91-101. https://doi.org/10.1007/978-3-030-39561-2_6

Hannan, T. J., & Hunstad, D. A. (2016). A murine model for escherichia coli urinary tract infection. *Methods in Molecular Biology*, *1333*, 159–175. https://doi.org/10.1007/978-1-4939-2854-5_14

Havranova, J., Cardio, S., Krinock, M., Widawski, M., Sluder, R., Kumar, A., Hippen, J., & Goel, H. (2020). Cranberry Extract for Preventing Recurrent Urinary Tract Infections: An Outcome-Specific Meta-Analysis of Prospective Trials. *Journal of Women's Health and Development*, *03*(03), 222–242. https://doi.org/10.26502/fjwhd.2644-28840033

Heinrich, M., Appendino, G., Efferth, T., Fürst, R., Izzo, A. A., Kayser, O., Pezzuto, J. M., & Viljoen, A. (2020). Best practice in research – Overcoming common challenges in phytopharmacological research. *Journal of Ethnopharmacology*, *246*, 112230. https://doi.org/10.1016/j.jep.2019.112230

Hemeg, H. A., Moussa, I. M., Ibrahim, S., Dawoud, T. M., Alhaji, J. H., Mubarak, A. S., Kabli, S. A., Alsubki, R. A., Tawfik, A. M., & Marouf, S. A. (2020). Antimicrobial effect of different herbal plant extracts against different microbial population. *Saudi Journal of Biological Sciences*, *27*(12), 3221–3227. https://doi.org/10.1016/j.sjbs.2020.08.015

Horváth, J., Wullt, B., Naber, K. G., & Köves, B. (2020). Biomarkers in urinary tract infections - which ones are suitable for diagnostics and follow-up? *GMS Infectious Diseases*, *8*, Doc24. https://doi.org/10.3205/id000068

Hsiao, C. Y., Chen, T. H., Lee, Y. C., Hsiao, M. C., Hung, P. H., Chen, Y. Y., & Wang, M. C. (2019). Urolithiasis Is a Risk Factor for Uroseptic Shock and Acute Kidney Injury in Patients With Urinary Tract Infection. *Frontiers in Medicine*, *6*(December), 1–7. https://doi.org/10.3389/fmed.2019.00288

Hung, C. S., Dodson, K. W., & Hultgren, S. J. (2009). A murine model of urinary tract infection. *Nature Protocols*, *4*(8), 1230–1243. https://doi.org/10.1038/nprot.2009.116

Ivanović, M., Razboršek, M. I., & Kolar, M. (2020). Innovative extraction techniques using deep eutectic solvents and analytical methods for the isolation and characterization of natural bioactive compounds from plant material. *Plants*, *9*(11), 1–29. https://doi.org/10.3390/plants9111428

Islam, R., Parves, M. R., Paul, A. S., Uddin, N., Rahman, M. S., Mamun, A. Al, Hossain, M. N., Ali, M. A., & Halim, M. A. (2021). A molecular modeling approach to identify effective antiviral phytochemicals against the main protease of SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics*, *39*(9), 3213–3224. https://doi.org/10.1080/07391102.2020.1761883

Jagtap, P., Bhise, K., & Prakya, V. (2015). A Phytopharmacological Review on Garcinia indica. *International Journal of Herbal Medicine*, *3*(4), 2–7.

Jeitler, M., Michalsen, A., Schwiertz, A., Kessler, C. S., Koppold-Liebscher, D., Grasme, J., Kandil, F. I., & Steckhan, N. (2022). Effects of a Supplement Containing a Cranberry Extract on Recurrent Urinary Tract Infections and Intestinal Microbiota: A Prospective, Uncontrolled Exploratory Study. *Journal of Integrative and Complementary Medicine*, *28*(5), 399–406. https://doi.org/10.1089/jicm.2021.0300

Jhang, J. F., Lin, T. Y., Ho, H. C., Jiang, Y. H., Hsu, Y. H., Birder, L. A., & Kuo, H. C. (2021). Deficits of urothelial cell proliferation, cytoskeleton, and barrier function protein expressions in patients with recurrent and persistent urinary tract infections. *LUTS: Lower Urinary Tract Symptoms*, *13*(2), 203–209. https://doi.org/10.1111/luts.12351

Jung, C., & Brubaker, L. (2019). The etiology and management of recurrent urinary tract infections in postmenopausal women. *Climacteric*, *22*(3), 242–249. https://doi.org/10.1080/13697137.2018.1551871

Karah, N., Rafei, R., Elamin, W., Ghazy, A., Abbara, A., Hamze, M., & Uhlin, B. E. (2020). Guideline for urine culture and biochemical identification of bacterial urinary pathogens in low-resource settings. *Diagnostics*, *10*(10). https://doi.org/10.3390/diagnostics10100832

Khan, M. A., Rahman, A. U., Khan, B., Al-Mijalli, S. H., Alswat, A. S., Amin, A., Eid, R. A., Zaki, M. S. A., Butt, S., Ahmad, J., Fayad, E., & Ullah, A. (2023). Antibiotic Resistance Profiling and Phylogenicity of Uropathogenic Bacteria Isolated from Patients with Urinary Tract Infections. *Antibiotics*, *12*(10). https://doi.org/10.3390/antibiotics12101508

Khapare, L. S., Kadam, J. H., & Shirke, G. D. (2020). *Garcinia a medicinally potential genus in Western Ghats*. *9*(5), 2750–2752.

Komal, S., Kazmi, S. A. J., Khan, J. A., & Gilani, M. M. (2018). Antimicrobial activity of prunella vulgaris extracts against multi-drug resistant Escherichia coli from patients of urinary tract infection. *Pakistan Journal of Medical Sciences*, *34*(3), 616–620. https://doi.org/10.12669/pjms.343.14982

Kumar, H., Insan, N. G., Chauhan, S., & Singh, V. A. (2017). Uropathogens: Isolation and antibacterial susceptibility pattern. *Asian Journal of Pharmaceutical and Clinical Research*, *10*(6), 222–224. https://doi.org/10.22159/ajpcr.2017.v10i6.18054

Kovacs, N. (1928). Z. ImmunFor.sch. Exp. Ther. 55, 31 1 -315

Li, X. Q., Zhu, J. Y., Pan, R. R., Shen, Y. L., Rahman, K., Zhang, C. Y., Zhang, L. J., Luan, X., & Zhang, H. (2019). Therapeutic effect of Dongbai-Tonglin-Fang, a Chinese herbal formula, on urinary tract infection in rat model. *Journal of Ethnopharmacology*, *241*(February), 112028. https://doi.org/10.1016/j.jep.2019.112028

Liang, J., Huang, X., & Ma, G. (2022). Antimicrobial activities and mechanisms of extract and components of herbs in East Asia. *RSC Advances*, *12*(45), 29197–29213. https://doi.org/10.1039/d2ra02389j

Lim, S. H., Lee, H. S., Lee, C. H., & Choi, C. I. (2021). Pharmacological Activity of Garcinia indica (Kokum): An Updated Review. *Pharmaceuticals*, *14*(12), 1–13. https://doi.org/10.3390/ph14121338

Lin, W. Y., & Liaw, S. J. (2020). Deacidification by FhlA - dependent hydrogenase is involved in urease activity and urinary stone formation in uropathogenic Proteus mirabilis. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-76561-w

Madeeha Mehboob, & Malik Nawaz Shuja. (2021). Isolation, Identification and Characterization of Urinary Tract Infectious Bacteria and the Effect of Different Antibiotics. *BioScientific Review*, *3*(3). https://doi.org/10.32350/bsr.0303.04

Madle, W., Ajeel, M., Alkataan, M., Ajeel, A., & Abd-alwahab, W. (2018). Anti-adhesion therapy, a promising alternative in the infections treatment. *Iraqi Journal of Pharmacy*, *15*(1), 46–60. https://doi.org/10.33899/iphr.2018.164196

Mazzariol, A., Bazaj, A., & Cornaglia, G. (2017). Multi-drug-resistant Gram-negative bacteria causing urinary tract infections: a review. *Journal of Chemotherapy*, *29*(December), 2–9. https://doi.org/10.1080/1120009X.2017.1380395

Mazziotti, M., Henry, S., Laval-Gilly, P., Bonnefoy, A., & Falla, J. (2018). Comparison of two bacterial DNA extraction methods from non-polluted and polluted soils. *Folia Microbiologica*, *63*(1), 85–92. https://doi.org/10.1007/s12223-017-0530-y

Medina, M., & Castillo-Pino, E. (2019). An introduction to the epidemiology and burden of urinary tract infections. *Therapeutic Advances in Urology*, *11*, 3–7. https://doi.org/10.1177/1756287219832172

Mollick, S., Dasgupta, T., Hasnain, J., & Ahmed, M. (2016). Isolation and characterization of pathogens responsible for urinary tract infection in Bangladesh and determination of their antibiotic susceptibility pattern. *Journal of Applied Pharmaceutical Science*, *6*(4), 72–76. https://doi.org/10.7324/JAPS.2016.60410

Moubayed, S., Ghazzawi, J., Mitri, R., & Khalife, S. (2023). Recent Data Characterizing the Prevalence and Resistance Patterns of FimH-producing Uropathogenic Escherichia coli Isolated from Patients with Urinary Tract Infections in North Lebanon. *Archives of Clinical Infectious Diseases*, *18*(4). https://doi.org/10.5812/archcid-135782

Murray, M. G., & Thompson, W. F. (1980). Nucleic Acids Research Nucleic Acids Research. *Methods*, *12*(21), 8235–8251.

Murthy, H. N., Dandin, V. S., Dalawai, D., Park, S. Y., & Paek, K. Y. (2019). Bioactive Compounds from Garcinia Fruits of High Economic Value for Food and Health. *Reference Series in Phytochemistry*, *January*, 1643–1670. https://doi.org/10.1007/978-3-319-78030-6_65

Nair, A., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *Journal of Basic and Clinical Pharmacy*, *7*(2), 27. https://doi.org/10.4103/0976-0105.177703

Narayan Swamy, S. N., Jakanur, R. K., & Sangeetha, S. R. (2022). Significance of C-reactive Protein Levels in Categorizing Upper and Lower Urinary Tract Infection in Adult Patients. *Cureus*, *14*(6). https://doi.org/10.7759/cureus.26432

O'Brien, V. P., Hannan, T. J., Nielsen, H. V., & Hultgren, S. J. (2016). Drug and Vaccine Development for the Treatment and Prevention of Urinary Tract Infections. *Microbiology Spectrum*, *4*(1). https://doi.org/10.1128/microbiolspec.uti-0013-2012

Öztürk, R., & Murt, A. (2020). Epidemiology of urological infections: a global burden. *World Journal of Urology*, *38*(11), 2669–2679. https://doi.org/10.1007/s00345-019-03071-4

Pappo, A., Gavish, R., Goldberg, O., Bilavsky, E., Bar-sever, Z., & Krause, I. (2020). *Hyponatremia in childhood urinary tract infection*. 6–9. 861-867. https://doi.org/10.1007/s00431-020-03808-z

Pardeshi, P. (2020). Prevalence of urinary tract infections and current scenario of antibiotic susceptibility pattern of bacteria causing UTI. *Indian Journal of Microbiology Research*, *5*(3), 334–338. https://doi.org/10.18231/2394-5478.2018.0070

Petca, R. C., Mareș, C., Petca, A., Negoiță, S., Popescu, R. I., Boț, M., Barabás, E., & Chibelean, C. B. (2020). Spectrum and antibiotic resistance of uropathogens in romanian females. *Antibiotics*, *9*(8), 1–16. https://doi.org/10.3390/antibiotics9080472

Prashanth, T., Ranganatha, V. L., Ramu, R., Mandal, S. P., Mallikarjunaswamy, C., & Khanum, S. A. (2021). Synthesis, characterization, docking study and antimicrobial activity of 2-(4-benzoylphenoxy)-1-[2-(1-methyl-1H-indol-3-yl)methyl)-1H-benzo[d]imidazol-1-yl] ethanone derivatives. *Journal of the Iranian Chemical Society*, *18*(10), 2741–2756. https://doi.org/10.1007/s13738-021-02230-y

Rădulescu, D., David, C., Turcu, F., Spătaru, D., Popescu, P., & Văcăroiu, I. (2020). Combination of cranberry extract and D-mannose possible enhancer of uropathogen sensitivity to antibiotics in acute therapy of urinary tract infections: Results of a pilot study. *Experimental and Therapeutic Medicine*, *3*, 3399–3406. https://doi.org/10.3892/etm.2020.8970

Rao Gajula, S. N., Talari, S., Chilvery, S., Godugu, C., & Sonti, R. (2023). A unique in vivo pharmacokinetic profile, in vitro metabolic stability and hepatic first-pass metabolism of garcinol, a promising novel anticancer phytoconstituent, by liquid chromatography–mass spectrometry . *RPS Pharmacy and Pharmacology Reports*, *2*(2), 1–10. https://doi.org/10.1093/rpsppr/rqad017

Rai, S. R., & Sayeed, A. (2022). *5. Kokum-The Superfood of India*. *November*.

Raksha, R., Srinivasa, H., & Macaden, R. S. (2003). Occurrence and characterisation of Uropathogenic Escherichia coli in urinary tract infections. *Indian Journal of Medical Microbiology*, *21*(2), 102–107. https://doi.org/10.1016/s0255-0857(21)03130-3

Ranjan, A., Sridhar, S. T. K., Matta, N., Chokkakula, S., & Ansari, R. K. (2017). Prevalence of UTI among Pregnant Women and Its Complications in Newborns. *Indian Journal of Pharmacy Practice*, *10*(1), 45–49. https://doi.org/10.5530/ijopp.10.1.10

Rodriguez-Mañas, L. (2020). Urinary tract infections in the elderly: A review of disease characteristics and current treatment options. *Drugs in Context*, *9*, 1–8. https://doi.org/10.7573/DIC.2020-4-13

Sangaonkar, G. M., & Pawar, K. D. (2018). Garcinia indica mediated biogenic synthesis of silver nanoparticles with antibacterial and antioxidant activities. *Colloids and Surfaces B: Biointerfaces*, *164*, 210–217. https://doi.org/10.1016/j.colsurfb.2018.01.044

Sarshar, M., Behzadi, P., Ambrosi, C., Zagaglia, C., Palamara, A. T., & Scribano, D. (2020). FimH and anti-adhesive therapeutics: A disarming strategy against uropathogens. *Antibiotics*, *9*(7), 1–16. https://doi.org/10.3390/antibiotics9070397

Scharf, B., Schmidt, T. J., Rabbani, S., Stork, C., Dobrindt, U., Sendker, J., Ernst, B., & Hensel, A. (2020). Antiadhesive natural products against uropathogenic E. coli: What can we learn from cranberry extract? *Journal of Ethnopharmacology*, *257*(April), 112889. https://doi.org/10.1016/j.jep.2020.112889

Schembri, M. A., Kjaergaard, K., Sokurenko, E. V., & Klemm, P. (2001). Molecular characterization of the Escherichia coli FimH adhesin. *Journal of Infectious Diseases*, *183*, S28–S31. https://doi.org/10.1086/318847

Schobert, R., & Biersack, B. (2019). Chemical and Biological Aspects of Garcinol and Isogarcinol: Recent Developments. *Chemistry and Biodiversity*, *16*(9). https://doi.org/10.1002/cbdv.201900366

Shi, J., Zhan, Z. S., Zheng, Z. S., Zhu, X. X., Zhou, X. Y., & Zhang, S. Y. (2023). Correlation of procalcitonin and c-reactive protein levels with pathogen distribution and infection localization in urinary tract infections. *Scientific Reports*, *13*(1), 1–8. https://doi.org/10.1038/s41598-023-44451-6

Simmons, J. S. (1926). A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. *The Journal of Infectious Diseases*, 209-214.

Soxhlet, F. V. (1879). Die gewichtsanalytische bestimmung des milchfettes. *Polytechnisches Journal*, 232(5), 461-465.

Sushma, B., Murali, R., Shamala, A., Yalamalli, M., Kashyap, B., & mutans. (2020). Antibacterial Activity of Herbal Extracts against Oral Bacteria: An Invitro Study. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN*, *19*(6), 22–29. https://doi.org/10.9790/0853-1906072229

Tabassum, H., Ali, M. N., Al-Jameil, N., & Khan, F. A. (2013). Evaluation of antibacterial potential of selected plant extracts on bacterial pathogens isolated from urinary tract infections. *International Journal of Current Microbiology and Applied Sciences*, *2*(10), 353–368.

Tharachand, C., Immanuel Selvaraj, C., & Abraham, Z. (2015). Comparative evaluation of anthelmintic and antibacterial activities in leaves and fruits of Garcinia cambogia (gaertn.) desr. and Garcinia indica (dupetit-thouars) choisy. *Brazilian Archives of Biology and Technology*, *58*(3), 379–386. https://doi.org/10.1590/S1516-8913201500062

Tuccinardi, T. (2021). What is the current value of MM/PBSA and MM/GBSA methods in drug discovery? *Expert Opinion on Drug Discovery*, *16*(11), 1233–1237. https://doi.org/10.1080/17460441.2021.1942836

Vachvanichsanong, P., McNeil, E. B., & Dissaneewate, P. (2021). Extended-spectrum beta-lactamase Escherichia coli and Klebsiella pneumoniae urinary tract infections. *Epidemiology & Infection,* 149, e12. https://doi.org/10.1017/S0950268820003015

Valdés-Tresanco, M. S., Valdés-Tresanco, M. E., Valiente, P. A., & Moreno, E. (2020). AMDock: a versatile graphical tool for assisting molecular docking with Autodock Vina and Autodock4. Biology Direct, 15(1), 1–12. https://doi.org/10.1186/s13062-020-00267-2

Vincent, J.G., Vincent, H.W. and Morton, J., 1944. Filter paper disc modification of the oxford cup penicillin determination. Proceedings of the society for Experimental Biology and Medicine, 55(3), pp.162-164.

Voges, O., & Proskauer, B. (1898). Beitrag zur Ernährungsphysiologie und zur Differentialdiagnose der Bakterien der hämorrhagischen Septicämie. *Zeitschrift für Hygiene und Infektionskrankheiten,* 28, 20-32.

White, A. N., Learman, B. S., Brauer, A. L., & Armbruster, C. E. (2021). Catalase activity is critical for Proteus mirabilis biofilm development, extracellular polymeric substance composition, and dissemination during catheter-associated urinary tract infection. *Infection and Immunity*, *89*(10). https://doi.org/10.1128/iai.00177-21

Wagenlehner, F. M. E., Bjerklund Johansen, T. E., Cai, T., Koves, B., Kranz, J., Pilatz, A., & Tandogdu, Z. (2020). Epidemiology, definition and treatment of complicated urinary tract infections. *Nature Reviews Urology*, *17*(10), 586–600. https://doi.org/10.1038/s41585-020-0362-4

Woese, C. R., & Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America*, *74*(11), 5088–5090. https://doi.org/10.1073/pnas.74.11.5088

Yu, L., & Shi, H. (2021). Recent advances in anti-adhesion mechanism of natural antimicrobial agents on fresh produce. *Current Opinion in Food Science*, 42, 8–14. https://doi.org/10.1016/j.cofs.2021.01.009

Zeka, K., Ruparelia, K. C., Continenza, M. A., Stagos, D., Vegliò, F., & Arroo, R. R. J. (2015). Petals of Crocus sativus L. as a potential source of the antioxidants crocin and kaempferol. *Fitoterapia*, *107*, 128–134. https://doi.org/10.1016/j.fitote.2015.05.014

Zhao, M., Lepak, A. J., & Andes, D. R. (2016). Animal models in the pharmacokinetic/pharmacodynamic evaluation of antimicrobial agents. *Bioorganic and Medicinal Chemistry*, *24*(24), 6390–6400. https://doi.org/10.1016/j.bmc.2016.11.008

Zhu, K., Hill, W. G., Li, F., Shi, B., & Chai, T. C. (2021). Urine and Tissue Bacterial Loads Correlate With Voiding Behaviors in a Murine Urinary Tract Infection Model. *Urology*, *154*, 344.e1-344.e7. https://doi.org/10.1016/j.urology.2021.05.009